particle production containing binding sites for such repressors and the use of vectors containing such binding sites for DNA delivery.

Finally, it is another object to reduce RCA in preparations of Ad virus by constructing such vectors and a helper virus with no overlap in the packaging sequences to eliminate homologous recombination.

SUMMARY OF THE INVENTION

The present invention relates to adenovirus vectors containing a minimum packaging signal for producing adenovirus virions. Of special importance is the presence of a CG dinucleotide located downstream of a TTTG sequence within each of the packaging elements. Spacing between the consensus segment 5'-TTTG-3' and the 5'-CG-3' segment located downstream is preferably between 1 and 12 nucleotides. Alternatively, it may be preferred to configure the consensus segments so that these elements appear on the same surface of the DNA helix. Most preferably, the adenovirus vector of the present invention may contain a packaging element consisting of 5'-TTTGN_8CG-3' (SEQ ID NO:1) which represents a minimal sequence necessary for adenovirus packaging. This sequence is preferably present in multiple copies. One type of minimal packaging sequence is an "A repeat", which contains a consensus sequence. Several A repeat sequences are shown in Table 1.

Another aspect of the present invention relates to novel vectors containing the minimum packaging sequences which can be selectively regulated. One such embodiment provides an adenovirus vector containing minimum packaging sequences and repressor

consensus segments so that the segments appear on the same surface of the DNA helix. The packaging element may be inserted into the left or right end of the adenovirus vector, preferably within 600 nucleotides from either end. More preferably, this minimal sequence is present at the left end of the genome and is present in multiple copies. Another consensus sequence comprises 5'-ATTTGN$_8$CG-3' (SEQ ID NO:2) and provides a strong packaging signal in adenovirus vectors. Two copies of this minimal packaging sequence are sufficient to ensure packaging. More than two copies enhance virus packaging. However, any number of this sequence can be inserted into the virus to ensure particle production. "Multimerized" as this term is used in the instant application refers to multiple copies of an element (i.e. packaging or repressing). These elements may be present in single units or in multimers, which preferably means 2-36 repeats and more preferably 2-12 units or elements. One form of the minimal packaging element is an "A repeat", which is derived from adenovirus. Representative A repeats are set forth below in Table 1:

TABLE 1

AI:	5'-TTTGGGCGTAACCG-3' (SEQ ID NO:3)
AII:	5'-TTTGGCCATTTTCG-3' (SEQ ID NO:4)
AIII:	5'-TCTGAATAATTTTG-3' (SEQ ID NO:5)
AIV:	5'-TTTGTGTTACTCAT-3' (SEQ ID NO:6)
AV:	5'-TTTGTCTAGGGCCG-3' (SEQ ID NO:7)
AVI:	5'-TTTGACCGTTTACG-3' (SEQ ID NO:8)
AVII:	5'-TTTACGTGGAGACT-3' (SEQ ID NO:9)

Unique adenovirus vectors that contain minimal packaging domains have been developed consisting of

EXAMPLES

MATERIALS AND METHODS

Virus constructions. Ad5 dl309, the parent for all the viruses described in this report, is a phenotypically wild type virus that contains a unique XbaI cleavage site at 3.8 map units (Jones, et al. (1979)). Plasmid pElA-194/814 contains the left end Ad5 XbaI fragment (nt 1-1339) with a deletion between nt 194 and 814 and a unique XhoI restriction site at the junction of the deletion. A head-to-tail hexamer of an oligonucleotide containing A repeat VI (5'-TCGACCGCGGGGACTTTGACC-3' (SEQ ID NO:10): 5'-TCGAGGTCAAAGTCCCCGCGG-3') (SEQ ID NO:11) was cloned into the 194/814 deletion. Similarly, head-to-tail hexamers of oligonucleotides containing A repeat I (5'-TCGAGTTGTAGTAAATTTGGG-3'(SEQ ID NO:12): 5'-TCGACCCAAATTTACTACAAC-3') (SEQ ID NO:13) or A repeat II (5'-TCGACCGAGTAAGATTTGGCC-3' (SEQ ID NO:14): 5'-TCGAGGCCAAATCTTACTCGG-3') (SEQ ID NO:15) were cloned into the pElA-194/814 background. pBR-194/814 and pBR-53/814 have sequences between nt 194 and 814 and nt 53 and 814 deleted. A monomer and dimer of viral sequences is located between nt 334 and 385 which contain AV, AVI, and AVII was cloned into the 194/814 deletion. A dimer of the nt 334 to 385 fragment as well as 12 head-to-tail copies of an oligonucleotide containing AVI (5'-TCGACCGCGGGGACTTTGACC-3'(SEQ ID NO:10): 5'-TCGAGGTCAAAGTCCCCGCGG-3') (SEQ ID NO:11) were cloned into the 53/814 deletion in either orientation. All mutations were verified by nucleotide sequence analysis.

at 10 V/cm on a 3.5% 30:1 (acrylamide: bisacrylamide) polyacrylamide gel in 0.5x TBE (25 mM Tris pH 8.3, 25 mM boric acid, 0.5 mM EDTA) at 4°C. For gel mobility shift assays performed with *in vitro* translated COUP-TFI protein, 0.25-1.5 µl of rabbit reticulocyte extract programmed with *in vitro* synthesized RNA transcript encoding COUP-TFI was assayed using the binding conditions described above. *In vitro* transcription and translation was performed as recommended by the manufacturer (Promega). For gel mobility supershift experiments, 0.5 µl of a rabbit polyclonal anti-COUP antiserum (a gift from Dr. Alonzo D. Garcia) was added after a one hour binding reaction, and incubation was then continued for an additional 30 minutes.

Plasmids, probes and competitor fragments. Head-to-tail hexamers of A repeats I and VI, individually, and a dimer of A repeats V-VII were cloned into pUC9. The sequence of a monomer of A repeat I is: 5'-TCGAGTTGTAGTAAATTTGGG-3': (SEQ ID NO:12): 5'-TCGACCCAAATTTACTACAAC-3' (SEQ ID NO:13), a monomer of A repeat VI is: 5'-TCGACCGCGGGGACTTTGACC-3' (SEQ ID NO:10): 5'-TCGAGGTCAAAGTCCCCGCGG-3' (SEQ ID NO:11). A monomer of AV-VII is: 5'-TCGACCGCGTAATATTTGTCTAGGGCCGCGGGGACTTTGACCGTTTACGTGGAGACTCC-3' (SEQ ID NO:16): 5'-TCGAGGAGTCTCCACGTAAACGGTCAAAGTCCCCGCGGCCCTAGACAAATATTACGCGG-3' (SEQ ID NO:17). The fragments were liberated from the vector by digestion with EcoRI and HindIII, gel purified and ^{32}P-end-labeled with Klenow DNA polymerase and (-^{32}P)dATP. For the preparation of ITR 1-13 probe, a monomeric oligonucleotide representing the

left end 13 nt flanked by Xho/Sal linkers 5'-TCGACATCAT CAATAATC-3'(SEQ ID NO:18): 5'TCGAGATTATTGATGATG (SEQ ID NO:19) was end-labeled in the same way using (α-^{32}P)dCTP.

For the preparation of competitor fragments containing packaging repeats, monomeric oligonucleotides were multimerized using T4 DNA ligase. Selection for head-to-tail multimers was achieved by subsequent digestion using SalI and XhoI, followed by phenol/ chloroform extraction and ethanol precipitation. In addition to multimers prepared from the oligonucleotides representing packaging elements I, VI and V-VII described above, A repeat II (5'TCGACCGAGTAAGATTTG GCC-3' (SEQ ID NO:14): 5'-TCGAGGCCAAATCTTACTCGG-3' (SEQ ID NO:15)) and A repeat V (5'-TCGACCGCGTAATATTTGTCC-3' (SEQ ID NO:20): 5'-TCGAGGACAAATATTACGCGG-3' (SEQ ID NO:21)) were used as multimeric competitors. Packaging repeat competitor fragments designated LS have the underlined nucleotides shown above in AI, AII, AV, AVI, AV-VI mutated into the sequence 5'GTGCAG-3' (only the upper strand is indicated). The italicized CG dinucleotide in the AV competitor was replaced by an AT in the competitor fragment designated CG. The competitor oligonucleotide representing ITR sequences 1-13 was used in monomeric form and was identical to the one used for probe preparation. The monomeric ITR 10-22 competitor oligonucleotide contains sequences between Ad nt 10-22 flanked by XhoI/SalI linkers. Quantitation of oligonucleotide competitors was performed spectrophotometrically. The amount of specific competitor DNA added per binding reaction is indicated in the text as -fold molar excess of binding sites present in the competitor relative to binding sites

RIGHT), P complex may consist of two distinct but interacting activities whereby one DNA binding activity binds the consensus A repeat sequence and the second DNA binding activity binds to the AT-rich left terminus of the adenovirus genome.

EXAMPLE 5

COUP-TF interacts with adenovirus packaging elements. Database searches revealed that the AVI probe contains highly conserved dimeric consensus binding sites for a cellular transcription factor, chicken ovalbumin upstream promoter transcription factor (COUP-TF; Cooney et al. (1992)). COUP-TF binds to the consensus sequence 5'-GGTCA-3' when situated as a direct or inverted repeat, with a preferred spacing of 1 base pair, and represented as perfect or imperfect versions of the consensus binding site. These binding sites overlap A repeat VI (5'-GGACTTTGACC-3' (SEQ ID NO:22); the COUP-TF inverted repeat is underlined, and AVI is in bold), only the upper strand is indicated with the COUP half sites underlined and AVI indicated in bold case. Other A repeats contain similar sequence motifs, albeit with less resemblance to the dimeric COUP consensus.

In view of the conserved COUP-TF binding motif contained within AVI, we asked whether the multimeric protein-DNA complexes formed on the AVI probe in particular, but also complexes formed on other A repeats, might contain COUP-TF (Schmid and Hearing, 1998). Heparin agarose fractions were subjected to Western blot analysis using a polyclonal COUP-TF antiserum. A band of approximately 45Kd molecular size was detected in fractions 24 to 31, which represents a